NeoBiota 56: 31–47 (2020)
doi: 10.3897/neobiota.56.47475
http://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Monitoring the silver carp invasion in Africa: a case study using environmental DNA (eDNA) in dangerous watersheds

Steven Crookes[1,2], Tej Heer[3], Rowshyra A. Castañeda[4,5], Nicholas E. Mandrak[3,4,5], Daniel D. Heath[1], Olaf L.F. Weyl[5,6], Hugh J. MacIsaac[1], Llewellyn C. Foxcroft[7,8]

1 *Great Lakes Institute for Environmental Research, University of Windsor, Ontario, N9B 3P4, Canada* **2** *Department of Integrative Biology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada* **3** *Department of Physical and Environmental Sciences, University of Toronto Scarborough, Toronto, Ontario, M1C 1A4, Canada* **4** *Department of Biological Sciences, University of Toronto Scarborough, Toronto, Ontario, M1C 1A4, Canada* **5** *DSI/NRF Research Chair in Inland Fisheries and Freshwater Ecology, South African Institute for Aquatic Biodiversity (SAIAB), Makhanda, Eastern Cape, South Africa, 6139, South Africa* **6** *Center for Invasion Biology, SAIAB, Makhanda, Eastern Cape, South Africa, 6139, South Africa* **7** *Conservation Services, South African National Parks, Kruger National Park, Skukuza 1350, South Africa* **8** *Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Matieland 7602, South Africa*

Corresponding author: Steven Crookes (scrookes@uoguelph.ca; stecrookes@googlemail.com)

Academic editor: M. Uliano-Silva | Received 20 October 2019 | Accepted 26 February 2020 | Published 29 April 2020

Citation: Crookes S, Heer T, Castañeda RA, Mandrak NE, Heath DD, Weyl OLF, MacIsaac HJ, Foxcroft LC (2020) Monitoring the silver carp invasion in Africa: a case study using environmental DNA (eDNA) in dangerous watersheds. NeoBiota 56: 31–47. https://doi.org/10.3897/neobiota.56.47475

Abstract

Biodiverse habitats are increasingly subject to an intensification of anthropogenic stressors that may severely diminish species richness. Invasive species pose a dominant threat to biodiversity and biosecurity, particularly in biodiversity hotspots like Kruger National Park, South Africa. The invasive silver carp, *Hypophthalmichthys molitrix*, was introduced into the Olifants River and may experience range spread owing to favorable environmental conditions. Intensive monitoring protocols are necessary to effectively manage invasions of species like silver carp. Unfortunately, tropical and sub-tropical aquatic systems are difficult to monitor using conventional methods (e.g., netting, electrofishing and snorkeling) owing to a range of factors including the presence of dangerous megafauna. Conservation of such systems may be advanced by the adoption of novel methods, including environmental DNA (eDNA) detection. Here, we explore the utility of environmental DNA (eDNA) to conduct safe, reliable and repeatable surveys in dangerous watersheds using silver carp as a case study. We conducted eDNA surveys at 12 sites in two neighbouring watersheds, and determined that the species has expanded its range within the Olifants River and to the

Copyright *Steven Crookes et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

south in the Sabie River. Expansion in the former is consistent with the presence of suitable spawning conditions. We discuss the implications of this survey for biodiversity monitoring in similar aquatic systems in the tropics and advocate an integrative approach to biomonitoring in these ecosystems.

Keywords
Biomonitoring, hazardous sampling, invasive species, Asian carp, species detection

Introduction

Africa is home to some of the most diverse habitats on the planet, encompassing myriad climatic, geologic and biotic zones (Happold and Lock 2012). This continent contains 22% of the highest-ranked watersheds supporting human populations, 9% of biodiverse hotspots, and is second to only central and southeast Asia in global importance for ecosystem services (Luck et al. 2009). In Africa and beyond, watersheds represent essential components of the socio-economic and cultural landscape, providing key ecosystem services, and are in need of effective and strategic management (Flotemersch et al. 2015).

River systems are threatened by direct modification through channelization (Emerson 1971), channel rerouting, construction of dams, weirs, locks and arterial canal networks, changes in drainage and overflow within the drainage basin (Johnson et al. 2009), and by the translocation of native species and introduction of non-indigenous species (Zhang et al. 2015). Poor watershed management can adversely affect nutrient and habitat availability, species distribution and abundance, population viability, and isolation, erosion or total loss of genetic variation (Davis et al. 2018). Poor management of fisheries and other biotic resources has resulted in introductions of detrimental non-indigenous taxa (i.e. aquatic invasive species; AIS) (Cucherousset and Olden 2011).

Monitoring the biotic component of river systems is essential to effective management of watersheds. For fishes, conventional monitoring of lotic habitats has traditionally relied upon methods including nets (e.g. seine, fyke, gill) or traps, angling, direct observation (SCUBA-diving or snorkeling), electrofishing, and telemetry and acoustic monitoring (Portt et al. 2006). In certain circumstances, however, these methods can be ineffective or too dangerous to deploy. Strong currents can preclude use of these methods or limit their utility to seasonal windows (Portt et al. 2006). Factors idiosyncratic to each method will also limit the scope and utility of each (e.g. low visibility will impact direct observation (Mueller et al. 2006)). In large areas of Africa, as well as Australia, southeast Asia and South America, the presence of large semi- or fully obligate aquatic mammals or reptiles represent a real danger to river researchers and affect surveys by destroying or interfering with equipment (World Health Organization 2003). In addition, the accidental bycatch and mortality of aquatic mammals and reptiles during fish surveys is a growing conservation concern (Ellender et al. 2016; Carrizo et al. 2017).

The Olifants River in southern Africa is home to dangerous aquatic megafauna animals including the common hippopotamus (*Hippopotamus amphibius* (Linnaeus, 1758)) and Nile crocodile (*Crocodylus niloticus* (Laurenti, 1768)) (Carrizo et al. 2017). Safe monitoring of river ecosystems containing all or some of this megafauna is highly problematic using conventional sampling tools. Environmental DNA (eDNA) detection was developed to indirectly detect a target species by collecting cellular and free aqueous DNA shed by the target species into the water column without the need for actual observation or collection of the organism itself (Ficetola et al. 2008; Thomsen and Willerslev 2015). By collecting water, the time and personnel required to conduct sampling are reduced (Thomas et al. 2019), thereby enhancing safety to both surveying personnel and co-occurring wildlife. In addition, accruing evidence indicates that eDNA detection may be more sensitive at detecting rare species than conventional methods (e.g. Dejean et al. 2012; Biggs et al. 2015).

Silver carp (*Hypophthalmichthys molitrix* (Valenciennes, 1844)) was first introduced to South Africa in 1975, when individuals from a German population were donated to the Marble Hall experimental fish farm adjacent to the Olifants River (Lübcker et al. 2014). It was suspected to have spread into the wider Olifants system, including the Olifants River, Lake Flag Boshielo (an impoundment on the Olifants River), and the Massingir dam, Mozambique (Sara et al. 2018), although the extent of its overall distribution remains uncertain. Lübcker et al. (2014) proposed that most of northeastern South Africa was suitable for colonization by this species. Here, we use eDNA to assess presence of silver carp in the Olifants River in Kruger National Park (South Africa) and Limpopo National Park (Mozambique) in southern Africa. We determined the ability to detect silver carp in the system using eDNA and comment on the utility of this method in the field to reduce potential human-wildlife conflict.

Methods

Study organism and sites

Silver carp is a highly invasive fish extensively introduced from its native range in eastern Asia to Europe, North America, and southeast Asia (DeGrandchamp et al. 2007). It is a highly effective filter feeder, consuming a range of microscopic forage that is channeled into high growth and fecundity rates (DeGrandchamp et al. 2007). Experimental and modeling data indicate that many populations of native North American fishes may be extirpated through direct competition or cascading trophic effects resulting from silver carp introduction, thus further spread in southern Africa is worrisome (Zhang et al. 2015).

We identified 12 sites in the Olifants watershed (Table 1; Figure 1) as suitable locations for sampling based upon site access and anecdotal evidence of carp capture, and to determine if a large hydroelectric dam protected the Upper Olifants from be-

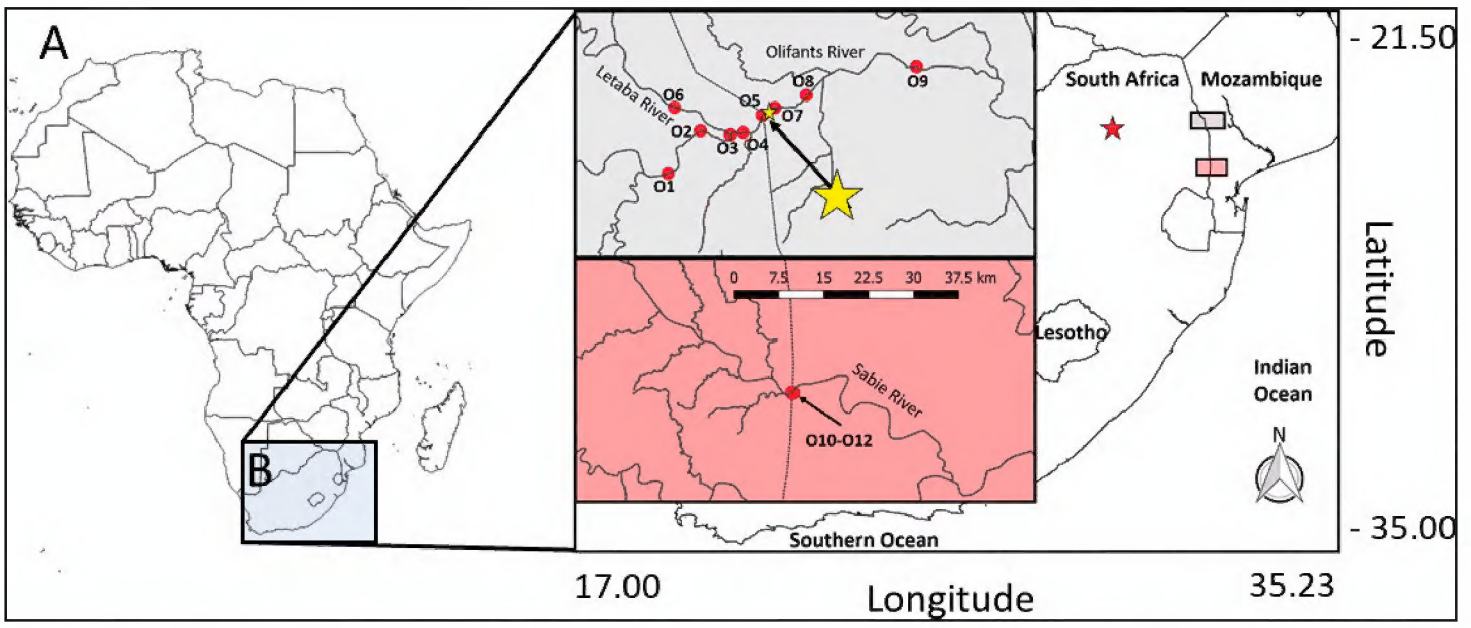

Figure 1. Map of sampling locations for silver carp eDNA within the continent of Africa (**A**). All sampled sites within southeast Africa (**B**) are shown as red circles. The two areas sampled are the Olifants system (Olifants and Letaba Rivers, grey inset) and the Komati (Sabie River, red inset). Also shown is the site of introduction and escape of silver carp in South Africa (red star) and the location of the Massingir dam, Mozambique (yellow star). Dotted line delimits the South Africa – Mozambique border.

Table 1. Sampling information and results of the qPCR analysis for the presence of silver carp eDNA at twelve sites in the Olifants watershed 'PCR' column indicates how many duplicate reactions for each of the three biological replicates were positive. The final column shows the mean Cq values across all positives (omitting non-amplifications) and their standard deviation (SD).

Site Code	Date	Site Description	River	Geographic Co-Ordinates	PCR	Mean Cq ± SD
O1	16/06/15	Olifants River Weir	Olifants	-24.055824, 31.720335	0/0/0	N/A
O2	16/03/15	East of Olifants Camp	Olifants	-23.982616, 31.775594	2/2/0	34.622 ± 0.688
O3	16/03/15	Olifants/Letaba Confluence	Olifants	-23.989445, 31.826483	2/0/1	31.744 ± 0.087
O4	17/03/16	Olifants River Gorge	Olifants	-23.985550, 31.848714	2/2/2	32.434 ± 1.267
O5	17/03/15	South Africa Border	Olifants	-23.956183, 31.881781	0/1/2	34.884 ± 1.737
O6	17/03/15	Letaba River Weir	Letaba	-23.942911, 31.731429	2/2/2	33.809 ± 4.149
O7	19/03/15	Upper Olifants, Above Dam	Olifants	-23.943567, 31.902952	2/0/2	33.914 ± 2.261
O8	19/03/15	Massingir Dam, Pelagic	Olifants	-23.921727, 31.956548	0/2/2	30.615 ± 2.161
O9	20/03/15	Massingir Dam Wall	Olifants	-23.873332, 32.145614	1/0/2	33.549 ± 0.639
O10	21/03/15	Coromana, Mozambique	Sabie	-25.184227, 32.033023	1/2/0	35.038 ± 0.091
O11	21/03/15	Coromana, South Africa	Sabie	-25.185171, 32.031348	2/2/1	34.238 ± 1.619
O12	21/03/15	Upper Sabie, South Africa	Sabie	-25.183838, 32.030184	2/0/1	36.208 ± 2.248

ing invaded from downstream sites. Three sites (O10–O12) were located in the Sabie River, part of the neighbouring Komati watershed, to determine if the carp has spread beyond the borders of the Olifants watershed.

Water collection, transportation and filtration

At each site, three 2 L water samples (3 × biological replicates) were collected in sterile (10% bleach solution (6% w/v sodium hypochloride)) polycarbonate plastic Nalgene

bottles. Unless access was difficult, all sampling was conducted by reaching from the bank to extract a sample from the top 5 cm of surface water in the littoral zone. Using single-use gloves for each sampling event, each sterile bottle was swept through the surface until filled. Each sample was immediately placed in a bleach-sterilized cooler and held at 4 °C during transportation back to the laboratory. Where direct access to the riverbank was difficult (e.g., large stretches with high embankments and prolific scrub vegetation), the site was accessed by boat and water was collected from as close to the shoreline as possible. At each site, a single Nalgene bottle containing 2 L distilled water (environment blank control) was opened and exposed to the environment before the top was resealed.

All water samples were filtered immediately upon return from the field in a central bleach-sterilized laboratory located in Skukuza, Kruger National Park, or in an ad-hoc, bleach-sterilized field laboratory near Massingir, Mozambique. Water was vacuum pumped through 1.2 µm pore glass fibre filters (47 mm diameter, VWR 696-filter). The filtration set-up included a tripartite manifold system of three funnels, each provisioned with a magnetic seal that securely clasped a filter between the funnel and the pump. Each biological replicate was filtered simultaneously in each of the three funnels (3 × filters per 2 L sample). After each sample was filtered, the entire apparatus and surrounding area was bleached sterilized, wiped with distilled water, and left to dry before proceeding with the next sample. After each filtration event, a separate set of sterile forceps was used to submerge each filter in a 2 ml Eppendorf tube containing 95% ethanol for storage at -20 °C. All samples were shipped to Canada for eDNA detection analysis.

eDNA extraction

eDNA extraction was performed in a dedicated extraction space. Using a protocol adapted from Dougherty et al. (2016), filters were cut into quarters and placed in tubes containing 20 µl 1 mm-diameter glass beads and 500 µl of modified (Coyne et al. 2005) CTAB (2% w/v cetyltrimethylammonium bromide, 2% w/v polyvinylpyrrolidone, 1.4M NaCl, 100mM Tris-HCL, 20nM EDTA) buffer pre-warmed to 65 °C in a heat block. Samples were homogenized using an unequal gravity FastPrep F120 homogenizer (Thermo Savant Instruments, Ltd) at 6.5 m sec^{-1} for two minutes and then incubated at 65 °C for two hours. 500 µl chloroform-isoamyl alcohol was added to each tube, mixed over-end for 5 minutes and centrifuged at 13,000 g for 15 minutes. The upper aqueous phase was transferred to a new tube containing 500 µl ice-cold isopropanol and 250 µl NaCl solution to precipitate out the DNA whilst incubating at -20 °C overnight. After incubation, the tubes were centrifuged again at 13,000 g for 15 minutes to allow DNA to pellet at the bottom. The supernatant was discarded and 200 µl ethanol wash added prior to vortexing and centrifugation at 13,000 g for three minutes. A second ethanol-washing round followed prior to carefully discarding the ethanol and drying the pellet in a vacufuge at maximum spin before being centrifuged for 10–15 minutes at 35 °C. The DNA pellet was re-

suspended in 100 µl 1X T.E. buffer and heated to 55 °C for ten minutes. After each filter had been extracted from the filter paper, the eluted DNA from each quarter filter paper was pooled into a single tube before decanting into 50 µL aliquots to be stored long-term at -20 °C.

eDNA quantitative real-time polymerase chain reaction (qPCR) detection

The Asian carp invasion in North America has resulted in development of numerous eDNA assays to detect all four problem species, including two in the genus *Hypophthalmichthys* (Jerde et al. 2013). Because only silver carp has been known to have been introduced into the Olifants River and surrounding systems, we chose the assay with the highest sensitivity to detect fish in the genus *Hypophthalmichthys* (Wozney and Wilson 2017). We chose the CTM primer set (Carp Taqman Multiplex 1), without using the Taqman probe, to increase sensitivity, designed and optimized in singleplex reactions (Wozney and Wilson 2017) to amplify both species. The original intent of this assay was to amplify both target species but discriminate based on differential probe binding (see Wozney and Wilson 2017 for assay specifications).

All qPCR reactions were performed in a laboratory with no previous history of Asian carp tissue or DNA samples. For each pooled eDNA extract, two duplicate technical replicates (PCR reactions) were performed. For all 12 sites, this tallied to 72 reactions in total, six per site/location and two per biological replicate. Alongside the target reactions (and environmental blank – one per location), two no-template controls were run to control for qPCR reagent/sample contamination. All reactions were performed on a single reaction plate, thereby eliminating inter-run variance in PCR results. All reaction volumes were 20 µl, consisting of 200 nM of each primer (0.4 µl), 10 µl of PowerUp SYBR green mastermix (Applied Biosystems, USA) and the remaining volume (9.2 µl) made up of eDNA extract providing the template for the reaction. To determine whether PCR inhibition may impede positive detection, we reassessed each sample using a separate internal positive control (IPC) assay that consisted of a primer and probe set that amplify a unique, and not found in nature, manufactured DNA sequence. We used the Taqman-probe based IPC developed by Gasparini et al. (2020) in which 200 copies of the artificial template were added to every reaction alongside 9.2 uL of the eDNA sample with Taqman Universal Mastermix II (Applied Biosystems, USA) in lieu of PowerUp SYBR Green. Inhibition was inferred to have occurred if the PCR Cq values were delayed by more than 1 Cq value relative to the Cq value of NTC control (i.e. pure water added as template). qPCR was performed on a 96-well CFX Thermal Cycler (BioRad) and run according to the published protocol (Wozney and Wilson 2017). PCR data were recorded in two ways: 1) successful amplification defined as the production of an amplification curve that passed a threshold of fluorescence; and, 2) of those reactions that amplified successfully, the Cq (quantification cycle) value, whose quantity is inversely proportional to the amount of silver carp DNA in the original sample, was documented.

Statistical analysis

We initially performed a post-hoc power analysis to confirm that the sampling design was sufficient to correctly reject the null hypothesis of no detection, thus boosting confidence in any negative finding. We used Olson et al.'s (2012) method:

$$Y_{i,j} = \Pi_{i=1}^{j}(1-\hat{d})$$

where y = probability of a false negative (upper boundary of 0.05 (alpha)), j = number of samples to be subject to qPCR, and $\hat{d}$ is the proportion of samples (out of three biological replicates) that yield ≥ 1 eDNA positive qPCR detection, to determine the predicted minimal number of samples necessary to achieve 95% power, predicated on our observed data.

Following the adoption of eDNA as a proxy of occupancy in habitat occupancy models (e.g. Schmidt et al. 2013), we performed a simple estimation of detection probability (p), site occupancy (Ψ) and the probability of detecting silver carp eDNA per sampling event (θ). As eDNA sampling is inherently hierarchical, Bayesian models predicting the three detection parameters are less vulnerable to increasing variability in the data than frequentist methods (Doll and Lauer 2014). Although occupancy modeling is most useful when co-estimating variables that may influence the detection of the organism by its proxy (i.e. via eDNA molecules), hierarchical models that estimate base values of each of the above parameters would result in more accurate values than would a naïve calculation based on naked data alone (Dorazio and Erickson 2018). To estimate these parameters, we performed the analysis using the R package 'eDNAoccupancy' (Dorazio and Erickson 2018) employing 500,000 Markov Chain Monte Carlo iterations after which model parameters were fitted (discarding first 10% as burn-in). Finally, an analysis of variance of Cq value across sites and by river system, which were encoded as categorical variables, was performed using the 'aov' function in R.

To assess if the Olifants River is suitable for silver carp spawning, a preliminary assessment was completed using methodology developed by Heer et al. (2019). The assessment determined whether a tributary is suitable to potential Asian carp spawning using a decision tree that categorized the river into four categories (not suitable, minimally suitable, moderately suitable, and highly suitable). It was based on growing degree-days (base 15 °C; GDD15) accumulated in a 12-month period spanning 2017–2018, an estimated hatching distance, and water temperature and velocity thresholds for spawning. There were five thresholds: 633 GDD15 within a year to achieve maturation, minimum water temperature of 17 °C to initiate spawning run, estimated distance to hatch is less than the unimpounded length of the river, flow velocity spike of 0.7 m/s to initiate spawning, and 900 GDD15 to trigger mass spawning. To complete the assessment, mean daily water temperature was obtained for the Mamba weir and mean daily velocity was estimated at the Balule weir based on weir dimensions and measured stage and discharge data. One year of temperature and velocity data were available, July 1, 2017 to June 30, 2018.

Results

Molecular eDNA detection

Every site except for O1 was positive for silver carp eDNA in at least one technical replicate across three biological replicates (Table 1). Apart from site O1, all locations were positive for silver carp eDNA and had detection levels ≥ 50 % (at least three out of six technical PCR replicates). Sites O4 and O6 had 100% detection across the board, although they did not have the lowest mean Cq value, which occurred at site O8 (Mean Cq = 30.62 ± 2.16). Overall mean Cq across all successful PCRs and all sites was 33.63 ± 2.39. All samples, apart from O3, O9 and O10, did not show qPCR inhibition as all IPC Cq values were within the margin of acceptability (i.e. all samples were within 1 Cq of the mean NTC IPC value of 30.63). In these cases, a PCR negative could be correctly interpreted as a lack of target eDNA molecules in the template volume. However, for sites O3, O9 and O10, the IPC did not amplify at all (Cq = 0), indicating complete inhibition of the IPC.

Detection analysis

The percentage of successful biological replicates yielding at least one detection ($\hat{d}$) was 69.40 %. Post-hoc power analysis revealed that the minimal predicted number of biological replicates per site should be two for this system, assuming 95% power, thus providing confidence that non-detection of silver carp at site O1 was unlikely due to a lack of appropriate sampling intensity. An occupancy analysis resulted in a high level of detection of silver carp in the study area. Estimated global probability of detection (p) was 0.849, inferred site occupancy (Ψ) was 0.892, and sample detection probability (θ) was 0.754. An analysis of variance indicated a near-significant effect of watershed and sampling site upon the levels of silver carp eDNA (F-value = 2.910, p = 0.0657; and F = 2.005, p = 0.0652, respectively) (Figure 2 and Suppl. material 1: Table S1). However, two-way ANOVA revealed that watershed was more important in determining silver carp occupancy than individual site effects (F-value = 3.298, p = 0.0494 and F-value = 1.682, p = 0.140 for River and Site, respectively). The concentrated aggregate of sampling sites on the more southerly Sabie River tended to have higher mean Cq values – and thus lower quantity of silver carp eDNA – than most of those in the northerly Olifants system in South Africa (Figure 2).

Spawning habitat analysis

Preliminary spawning assessment indicates that the Olifants River was a minimally appropriate habitat in 2017–2018 (Figure 3). The river was always above the minimum 17 °C temperature, but never achieved flow sufficient to reach the higher levels of suit-

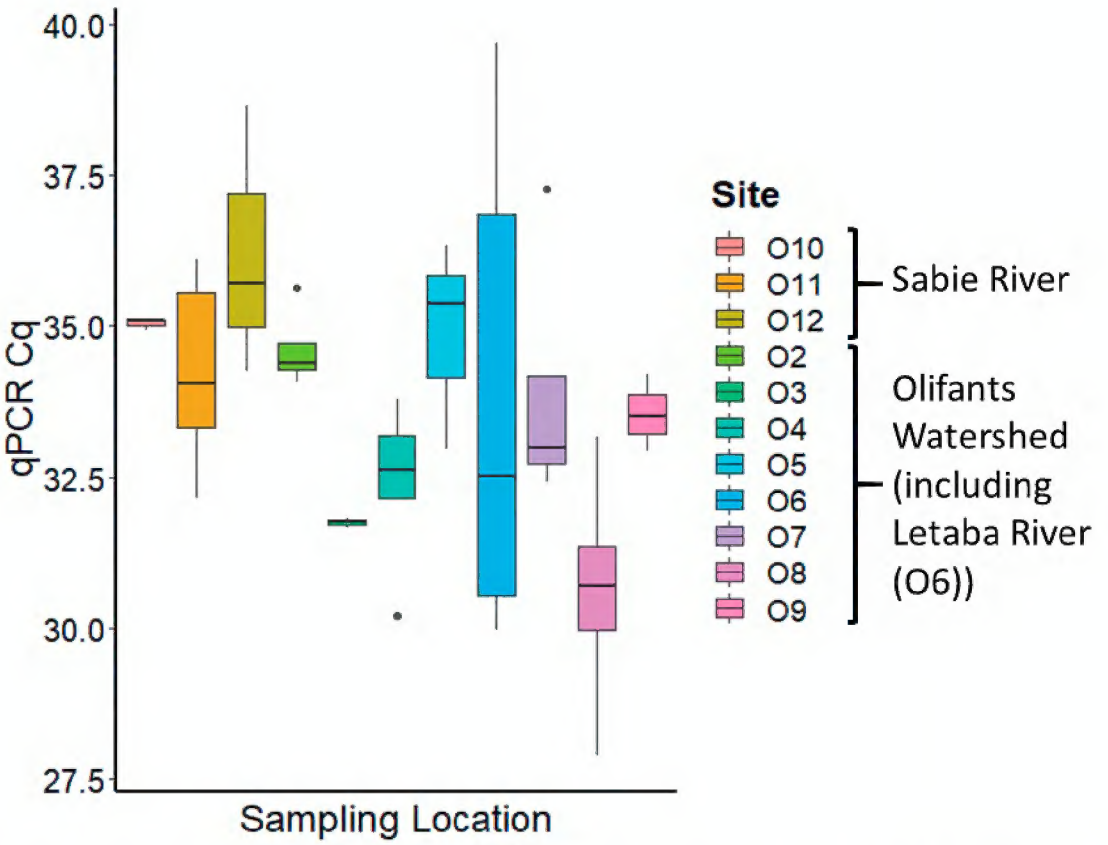

Figure 2. Levels and variance of qPCR detection (y-axis) of silver carp DNA among sampling sites within and between rivers in the Olifants system and Sabie River (x-axis).

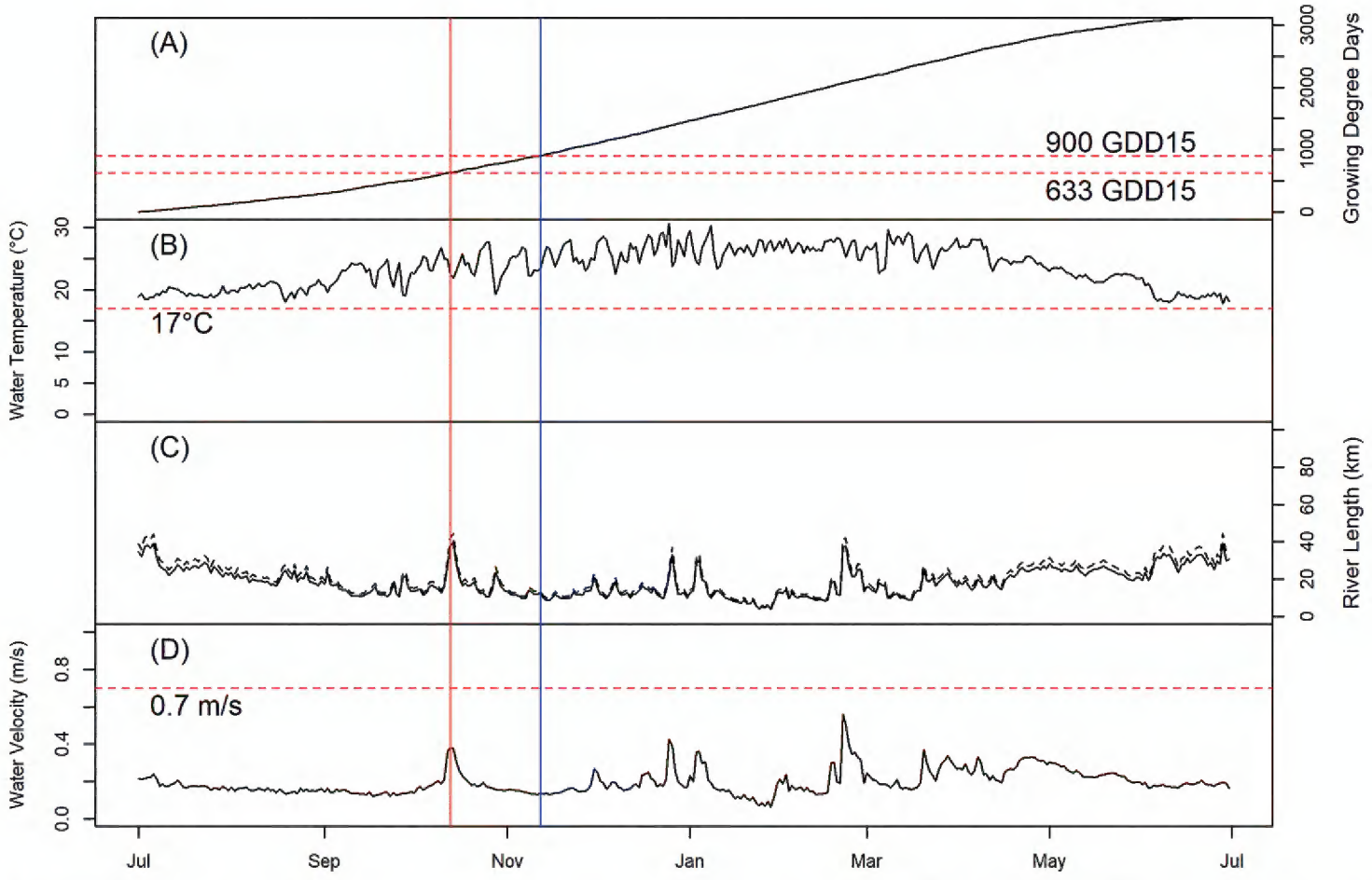

Figure 3. Results of a silver carp spawning suitability model for the Olifants River, July 1, 2017-June 30, 2018 **A** growing degree days (base 15). 633 GDD15 (red dashed line and solid red vertical line) is required for maturation and 900 GDD15 (blue dashed line and solid blue vertical line) is required for initiation of mass spawning **B** mean daily water temperate. Minimum temperature required (red dashed line) is always exceeded **C** unimpeded river length required for egg hatching to occur **D** mean daily water velocity. Flow spike required for high suitability is not achieved.

ability. Based on a July 1 winter start date, 633 GDD15 would have been achieved by early October and 900 GDD15 by early November. Following these spawning initiation dates, the length of unimpeded river required for egg hatching ranged 15–40 km, which is readily available in the Olifants River.

Discussion

Silver carp range expansion as inferred from eDNA presence data

Environmental DNA detection confirmed the presence of silver carp eDNA throughout the sampled areas of the Olifants River and neighbouring systems (where sampled), except for Site O1. Our study adds to the mounting evidence confirming this technique as a sensitive tool to monitor highly invasive Asian carps generally (Jerde et al. 2011, 2013; Turner et al. 2015; Wozney and Wilson 2017) and silver carp in particular (Amberg et al. 2015; Coulter et al. 2019; Stepien et al. 2019). Our study is the first to use a targeted eDNA approach to monitor for the presence of Asian carps on the African continent. However, the detection of silver carp was not without impediment, as the IPC results indicate that, in some sections of the Olifants and Sabie rivers, low levels of eDNA were beyond the level of sensitivity of the assay. The IPC was set at 200 copies per reaction, so even slight inhibition was detected. As many eDNA species targets are rare and shed low levels of eDNA, PCR inhibition is a potentially huge issue for delineation of the invasion front and detection of rare species where low abundances should translate into low eDNA levels and, possibly, false negatives. However, as we observed 3/6 silver carp qPCR detections for each of the IPC inhibited sites, we can conclude that, although silver carp eDNA levels were lower than at the other sites (except O1), they often exceed 200 copies per 9.2 µL (qPCR template volume) and are thus well within the level of detectability of this assay. As PCR inhibitors are present in the systems of study, it is likely that silver carp Cq values were delayed, if not outright inhibited, and thus it would be difficult to translate Cq values to copy numbers. Moreover, silver carp eDNA levels exhibit a nonlinear relationship with carp density (Coulter et al. 2019).

eDNA detection levels were high (except Site O1), with at least half of all qPCR reactions positive for silver carp. Notwithstanding the likely nonlinear relationship between eDNA levels and population densities, the fact that silver carp was detected in half of all qPCR reactions at three sites for which the IPC (200 copies per reaction) was completely inhibited is consistent with a large, established population constantly shedding a lot of eDNA into the surrounding environs. These results indicate that silver carp is pervasive in the Olifants watershed, supporting the proposition that this species is established within South Africa (Ellender and Weyl 2014). The data confirm the expansion of the silver carp range into Kruger National Park from elsewhere in the Olifants system, either from upstream at the Flag Boshielo Dam, Mpumalanga (Britts 2009) or from downstream (Schneider 2003). The data also confirm that the Massingir

population is still extant and healthy and corroborates local reports of the successful fishing of this species for local consumption. It is unlikely that escapees, or the intermittent use of silver carp as a baitfish, would yield a similar pattern of strong detections. All field controls were negative, thereby discounting the possibility of spurious results stemming from contamination as a factor responsible for positive detection results.

One criticism of eDNA utility in lotic systems, however, is the potential systemic bias introduced by the directional flow of water. Until recently, the conveyance of eDNA downstream was thought to operate primarily on relatively small spatial scales (up to 12 km) varying by hydrology, season and target organism (e.g. Deiner and Altermatt 2014; Shogren et al. 2017). Interestingly, Pont et al. (2018) reported eDNA transport distances of over 100 km in the voluminous River Rhône, France. However, if all positive eDNA tests in the upper Olifants River resulted from advection from a single upstream source, one would expect the eDNA signal to attenuate (e.g. show rising Cq values) with increasing distance from that source, albeit at low flow conditions (Jane et al. 2015). We observed no such trend with our data. The silver carp eDNA signal was strong above and below Massingir Dam, a large reservoir of the Olifants River on the border of South Africa and Mozambique. This is consistent with Schneider (2003), who reported silver carp invading the Massingir Dam in 1996/1997 and dispersing downstream into the mainstream Limpopo River during floods in 2000 (Schneider 2003). Natural dispersal events may also be supplemented by accidental and intentional anthropogenic release of larval and juvenile carp, which are hard to identify during early stages of ontogeny and which may be used as baitfish. In the Great Lakes region of North America, juvenile silver carp have been positively identified using qPCR as a part of the selection of baitfish available to anglers (Stepien et al. 2019).

Affirmation of habitat suitability for establishment of silver carp

Due to the long unimpounded distance of the Olifants River upstream of Massingir Dam, along with high temperatures, this system is suitable for silver carp spawning. Temperature data in the river indicate that maturation can occur by early October and mass spawning by early November based on a July 1 winter start date. However, silver carp maturity can be reached within approximately three months, indicating that spawning could potentially occur at any point in the year. The minimum temperature required for spawning (17 °C) was always met in this system. There exists some uncertainty with this approach, primarily due to the estimate of velocity based on weir dimensions and the assumption of linearity in the hatching distance.

Our findings are consistent with those derived from ecological niche modelling, in which the middle-lower Olifants River was assessed to have a high predicted climatic suitability for silver carp establishment (>75%) (Lübcker et al. 2014). However, the Komati watershed lies in an area predicted to have 80–100% suitability for the establishment of the silver carp. The modelling result is consistent with our observation of eDNA in the Sabie River, albeit at lower levels than in the Olifants. Interestingly, silver

carp has yet to be visually observed here, thus eDNA detection would be the first record of its occupancy in the Sabie River. The discrepancy in eDNA levels between the two watersheds is likely a function of the time since invasion. Silver carp has occupied the Olifants River at least since escaping captivity in the upper reaches of the river in 1992 (Britts 2009). Assuming the eDNA signal to be redolent of occupancy of silver carp in the Sabie River, it may be that the species has yet to establish and expand its range, although such an event may be anticipated if invasion follows a stepping-stone model of expansion (Alharbi and Petrovskii 2019). Indeed, the large floods caused by Cyclone Eline (UNDP 2000) resulted not only in the flooding of several fish farms containing silver carp in the lower Limpopo River floodplain in Mozambique, but also linked the Limpopo and Komati Rivers in March 2000 (Schneider 2003).

eDNA detection as a tool for aquatic biomonitoring in dangerous systems

The adoption of eDNA detection methods is especially pertinent in areas in which conventional monitoring can be inefficient or dangerous. These areas are often in the tropics, and harbor much of the world's biodiversity, including charismatic freshwater megafauna (Carrizo et al. 2017). At the time of our survey (2015), we adopted best practices for that time. This included taking physical water samples from the surface of water in close proximity to a range of unpredictable predators (Nile crocodile) and large herbivores (hippopotamus), which, while not without risk, was safer than deploying and collecting nets or physically entering the water. This also reduced the risk of accidental by-catch and accidental mortality of conservation priority taxa such as Nile crocodile, which are susceptible to entanglement and drowning in sampling gear such as gill nets (Ellender et al. 2016). As such, using eDNA to successfully detect the silver carp validates this approach for use in dangerous systems. Using eDNA lowers the risk of loss of equipment and time to pernicious and unpredictable events in the field. Dangerous systems may not just be limited to those containing large animals but also those with high levels of pollutants or pathogens, strong tidal or convectional currents, or rapids and unpredictable, seasonal flows. In the intervening time period, onsite eDNA developments have progressed so rapidly that much of the physical workflow is automated and can be operated remotely, including performing onsite qPCR detection (Thomas et al. 2018, 2019). Onsite methods also have the advantage of reducing the risk of laboratory-based contamination and the degradation of rare eDNA molecules in transit (attenuating both Types I and II error). Thus, eDNA detection methodology is suitable for highly biodiverse and remote areas such as Kruger National Park.

The success of this pilot project to investigate the utility of using eDNA detection to identify the presence of the silver carp in two watersheds in southern Africa cannot be disputed, at least for AIS. However, at-risk species (SAR) show similarities with invasion-front species in that often their *a priori* distribution is unknown or merely suspected, and whose populations are fragmented and characterized by few individuals. Yet, eDNA has been shown to be just as effective at targeting SAR as it has AIS (e.g. Balasingham et al. 2018; Currier et al. 2018). Furthermore, the

molecular methods used to conduct eDNA monitoring may also be extended. For example, qPCR-based tools can also be used to screen eggs to confirm spawning success in suitable habitat identified by *a posteriori* modelling or previous eDNA detection (Fritts et al. 2019).

Conclusion

We recommend that eDNA detection be used as part of the conservation biologist's toolbox when considering the management of invaders in dangerous aquatic ecosystems in the tropics and elsewhere. Moreover, future investigations should take into account the complexities of hydrodynamics when monitoring rivers, potentially by using hydrodynamic models (e.g. Garcia et al. 2013), which could be integrated with eDNA data (e.g. Carroro et al. 2018). Our results indicate that the Olifants River is suitable for silver carp spawning and could reach high suitability if it coincided with a flow spike that exceeds 0.7 m/s. These projections were validated by the eDNA detection data. We highlight the combined power of eDNA detection and habitat modelling tools to predict not only current distribution – and habitat suitability – of a target organism, but to also forecast which areas are at risk of imminent invasion. Adopting an integrative methodology, combining aspects of molecular, theoretical and field ecology to better effectively manage extremely limited resources is beneficial to optimizing efforts to conserve important refuges of aquatic biodiversity in the coming decades.

Acknowledgements

We thank Pauli Viljoen for logistical and field support. We thank South African National Parks for financial and logistical support. OLFW acknowledges support received through the National Research Foundation – South African Research Chairs Initiative of the Department of Science and Innovation (Grant No. 110507). The CIB/DSI Centre of Excellence for Invasion Biology (CIB) for continued support. LCF acknowledges support from the DSI-NRF Centre of Excellence for Invasion Biology, Dept. of Botany and Zoology, Stellenbosch University, and the National Research Foundation (of South Africa, Project Numbers IFR2010041400019 and IFR160215158271). NEM, DDH and HJM were supported by NSERC Discovery grants and the NSERC CAISN Strategic Network, while HJM also was supported by a Canada Research Chair in Aquatic Invasive Species.

References

Alharbi W, Petrovskii S (2019) Effect of complex landscape geometry on the invasive species spread: Invasion with stepping stones. Journal of Theoretical Biology 464: 85–97. https://doi.org/10.1016/j.jtbi.2018.12.019

Amberg JJ, McCalla SG, Monroe E, Lance R, Baerwaldt K, Gaikowski MP (2015) Improving efficiency and reliability of environmental DNA analysis for silver carp. Journal of Great Lakes Research 41: 367–373. https://doi.org/10.1016/j.jglr.2015.02.009

Balasingham KD, Walter RP, Mandrak NE, Heath DD (2018) Environmental DNA detection of rare and invasive fish species in two Great Lakes tributaries. Molecular Ecology 27: 112–127. https://doi.org/10.1111/mec.14395

Biggs J, Ewald N, Valentini A, Gaboriaud C, Dejean T, Griffiths RA, Wilkinson JW, Arnell A, Brotherton P, Williams P, Dunn F (2015) Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). Biological Conservation 183: 19–28. https://doi.org/10.1016/j.biocon.2014.11.029

Britts DL (2009) Distribution of the silver carp, *Hypopthalmichthys molitrix* (Valciennes, 1844) in the Flag Boshielo Dam (Arabie Dam) and its potential influences on the ecology of the dam. PhD Thesis, University of Limpopo, South Africa.

Carraro L, Hartikainen H, Jokela J, Bertuzzo E, Rinaldo A (2018) Estimating species distribution and abundance in river networks using environmental DNA. Proceedings of the National Academy of Sciences 115: 11724–11729. https://doi.org/10.1073/pnas.1813843115

Carrizo SF, Jähnig SC, Bremerich V, Freyhof J, Harrison I, He F, Langhans SD, Tockner K, Zarfl C, Darwall W (2017) Freshwater megafauna: flagships for freshwater biodiversity under threat. BioScience 67: 919–927. https://doi.org/10.1093/biosci/bix099

Coulter DP, Wang P, Coulter AA, Van Susteren GE, Eichmiller JJ, Garvey JE, Sorensen PW (2019) Nonlinear relationship between Silver Carp density and their eDNA concentration in a large river. PLoS ONE 14(6): e0218823. https://doi.org/10.1371/journal.pone.0218823

Coyne KJ, Handy SM, Demir E, Whereat EB, Hutchins DA, Portune KJ, Doblin MA, Cary SC (2005) Improved quantitative real-time PCR assays for enumeration of harmful algal species in field samples using an exogenous DNA reference standard. Limnology and Oceanography Methods 3: 381–391. https://doi.org/10.4319/lom.2005.3.381

Cucherousset J, Olden JD (2011) Ecological impacts of nonnative freshwater fishes. Fisheries 36(5): 215–230. https://doi.org/10.1080/03632415.2011.574578

Currier CA, Morris TJ, Wilson CC, Freeland JR (2018) Validation of environmental DNA (eDNA) as a detection tool for at-risk freshwater pearly mussel species (Bivalvia: Unionidae). Aquatic Conservation: Marine and Freshwater Ecosystems 28: 545–558. https://doi.org/10.1002/aqc.2869

Davis CD, Epps CW, Flitcroft RL, Banks MA (2018) Refining and defining riverscape genetics: how rivers influence population genetic structure. Interdisciplinary Reviews: Water 5(2): e1269. https://doi.org/10.1002/wat2.1269

Deiner K, Altermatt F (2014) Transport distance of invertebrate environmental DNA in a natural river. PLoS ONE 9(2): e88786. https://doi.org/10.1371/journal.pone.0088786

DeGrandchamp KL, Garvey JE, Cosboth LA (2007) Linking adult reproduction and larval density of invasive carp in a large river. Transactions of the American Fisheries Society 136: 1327–1334. https://doi.org/10.1577/T06-233.1

Dejean T, Valentini A, Miquel C, Taberlet O, Bellemain E, Miaud C (2012) Improved detection of an alien invasive species through environmental DNA barcoding: the example of

the American bullfrog *Lithobates catesbeianus*. Journal of Applied Ecology 49: 953–959. https://doi.org/10.1111/j.1365-2664.2012.02171.x

Doll JC, Lauer TE (2014) Comparing Bayesian and frequentists methods of fisheries models: hierarchical catch curves. Journal of Great Lakes Research 40: 41–48. https://doi.org/10.1016/j.jglr.2014.07.006

Dorazio RM, Erickson RA (2018) Ednaoccupancy: An r package for multiscale occupancy modelling of environmental DNA data. Molecular Ecology Resources 18: 368–380. https://doi.org/10.1111/1755-0998.12735

Dougherty MM, Larson ER, Renshaw MA, Gantz CA, Egan SP, Erickson DM, Lodge DM (2016) Environmental DNA (eDNA) detects the invasive rusty crayfish *Oronectes rusticus* at low abundances. Journal of Applied Ecology 53: 722–732. https://doi.org/10.1111/1365-2664.12621

Ellender BR, Weyl OLF (2014) A review of current knowledge, risk and ecological impacts associated with non-native freshwater fish introductions in South Africa. Aquatic Invasions 9: 117–132. https://doi.org/10.3391/ai.2014.9.2.01

Emerson JW (1971) Channelization: a case study. Science 173: 325–326. https://doi.org/10.1126/science.173.3994.325

Ficetola GF, Miaud C, Pompanon F, Taberlet P (2008) Species detection using environmental DNA from water samples. Biology Letters 4: 423–425. https://doi.org/10.1098/rsbl.2008.0118

Flotemersch JE, Leibowitz SG, Hill RA, Stoddard JL, Thoms MC, Tharme RE (2015) A watershed integrity definition and assessment approach to support strategic management of watersheds. River Research and Applications 32(7): 1654–1671. https://doi.org/10.1002/rra.2978

Fritts AK, Knights BC, Larson JH, Amberg JJ, Merkes, CM, Tajjioui T, Butler SE, Diana MJ, Wahl DH, Weber MJ, Waters JD (2019) Development of a quantitative PCR method for screening ichthyoplankton samples for bighead carps. Biological Invasions 21: 1143–1153. https://doi.org/10.1007/s10530-018-1887-9

Garcia T, Jackson PR, Murphy EA, Valocchi AJ, Garcia MH (2013) Development of a Fluvial Egg Drift Simulator to evaluate the transport and dispersion of Asian carp eggs in rivers. Ecological Modelling 263: 211–222. https://doi.org/10.1016/j.ecolmodel.2013.05.005

Gasparini L, Crookes S, Prosser R, Hanner RH (2020) Detection of Freshwater Mussels (Unionidae) using Environmental DNA in Riverine Systems. Environmental DNA: 1–9. https://doi.org/10.1002/edn3.71

Happold D, Lock MJ (2012) Mammals of Africa (Vol. I). Bloomsbury.

Heer T, Wells MG, Mandrak NE. (2019). Assessment of Asian carp spawning potential in tributaries to the Canadian Lake Ontario basin. Journal of Great Lakes Research 45: 1332–1339. https://doi.org/10.1016/j.jglr.2019.09.019

Jane SF, Wilcox TM, McKelvey KS, Young MK, Schwartz MK, Lowe WH, Letcher BH, Whiteley AR (2014) Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. Molecular Ecology Resources 15(1): 216–227. https://doi.org/10.1111/1755-0998.12285

Jerde CL, Mahon AR, Chadderton WL, Lodge DM (2011) "Sight-unseen" detection of rare aquatic species using environmental DNA. Conservation Letters 4(2): 150–157. https://doi.org/10.1111/j.1755-263X.2010.00158.x

Jerde CL, Chadderton WL, Mahon AR, Renshaw MA, Corush J, Budny M, Mysorekar S, Lodge DM (2013) Detection of Asian carp DNA as part of a Great Lakes basin-wide

surveillance program. Canadian Journal of Fisheries and Aquatic Sciences 70: 522–526. https://doi.org/10.1139/cjfas-2012-0478

Johnson AC, Acreman, AC, Dunbar MJ, Fesist SW, Giacomello AM, Gozlan RE, Hinsley SA, Ibbotson AT, Jarvie HP, Jones JI, Longshaw M, Maberly SC, Marsh TJ, Neal C, Newman JR, Nunn MA, Pickup RW, Reynard NS, Sullivan CA, Sumpter JP, Williams RJ (2009) The British river of the future: How climate change and human activity might affect two contrasting river ecosystems in England. Science of the Total Environment 407: 4787–4798. https://doi.org/10.1016/j.scitotenv.2009.05.018

Kolar CS, Chapman D, Courtenay W, Williams J, Jennings D (2007) Bigheaded Carps: a Biological Synopsis and Environmental Risk Assessment, Special publication 33. American Fisheries Society, Bethesda, 204 pp.

Lübcker N, Zengeya TA, Dabrowski J, Robertson MP (2014) Predicting the potential distribution of invasive silver carp *Hypophthalmichthys molitrix* in South Africa. African Journal of Aquatic Sciences 39: 157–165. https://doi.org/10.2989/16085914.2014.926856

Luck GW, Chan KMA, Fay JP (2009) Protecting ecosystem services and biodiversity in the world's watersheds. Conservation Letters 2(4): 179–188. https://doi.org/10.1111/j.1755-263X.2009.00064.x

Mueller RP, Brown RS, Hop H, Moulton L (2006) Video and acoustic camera techniques for studying fish under ice: a review and comparison. Review of Fish Biology and Fisheries 16: 213–226. https://doi.org/10.1007/s11160-006-9011-0

Olson ZH, Briggler JT, Williams RN (2012) An eDNA approach to detect eastern Hellbenders (*Cryptobranchus a. alleganiensis*) using samples of water. Wildlife Research 39: 629–636. https://doi.org/10.1071/WR12114

Pont D, Rocle M, Valentini A, Civade R, Jean P, Maire A, Roset N, Schabuss M, Zornig H, Dejean T (2018) Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. Scientific Reports 8: 1–13. https://doi.org/10.1038/s41598-018-28424-8

Portt CB, Coker GA, Ming DL, Randall RG (2006) A Review of Fish Sampling Methods Commonly used in Canadian Freshwater Habitats. Canadian Technical Report of Fisheries and Aquatic Sciences 2604, 51 pp.

Sara JR, Marr SM, Chabalala NM, Smit WJ, Erasmus LJC, Luus-Powell WJ (2018) Human health risks of metalloids and metals in muscle tissue of silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844) from Lake Flag Boshielo, South Africa. African Journal of Aquatic Science 43: 405–411. https://doi.org/10.2989/16085914.2018.1538869

Schmidt BR, Kéry M, Ursenbacher S, Hyman OJ, Collins JP (2013) Site occupancy models in the analysis of environmental DNA presence/absence surveys: a case study of an emerging amphibian pathogen. Methods in Ecology and Evolution 4(7): 646–653. https://doi: https://doi.org/10.1111/2041-210X.12052

Schneider MF (2003) Ocorrencia das Especies Comerciais Exoticasde Peixe no Rio Limpopo depois das Cheias no Ano 2000. Bole-tim de Investigacao Florestal (Materia Prima) 3: 31–36.

Shogren AJ, Tank JL, Andruszkiewicz E, Olds B, Mahon AR, Jerde CL, Bolster D (2017) Controls on eDNA movement in streams: transport, retention and resuspension. Scientific Reports 7: 1–11. https://doi.org/10.1038/s41598-017-05223-1

Stepien CA, Snyder MR, Elz AE (2019) Invasion genetics of the silver carp *Hypopthalmichthys molitrix* across North America: Differentiation of fronts, introgression, and eDNA metabarcode detection. PLoS ONE 14(3): e0203012. https://doi.org/10.1371/journal.pone.0203012

Thomas AC, Tank S, Nguyen PL, Ponce J, Sinnesael M, Goldberg CS (2019) A system for rapid eDNA detection of aquatic invasive species. Environmental DNA: 1–10. https://doi.org/10.1002/edn3.25

Thomas AC, Howard J, Nguyen PL, Seimon TA, Goldberg CS (2018) ANDeTM: a fully integrated environmental DNA sampling system. Methods in Ecology and Evolution 9(6): 1379–1385. https://doi.org/10.1111/2041-210X.12994

Thomsen PF, Willerslev E (2015) Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. Biological Conservation 183: 4–18. https://doi.org/10.1016/j.biocon.2014.11.019

Turner CR, Uy KL, Everhart RC (2015) Fish environmental DNA is more concentrated in aquatic sediments than surface water. Biological Conservation 183: 93–102. https://doi.org/10.1016/j.biocon.2014.11.017

World Health Organization (2003) Guidelines for safe recreational water environments (Vol. 1). Coastal and fresh waters. World Health Organization, Geneva, 253 pp.

Wozney KM, Wilson CC (2017) Quantitative PCR multiplexes for simultaneous multispecies detection of Asian carp eDNA. Journal of Great Lakes Research 43: 771–776. https://doi.org/10.1016/j.jglr.2017.05.001

United Nations in Mozambique (2001) [United Nations, Maputo (2000)] Assistance to Mozambique Following the Devastating Floods: Report of the Secretary General. A/55/123-E/2000/89. UN General Assembly, New York.

Zhang H, Rutherford ES, Mason DM, Breck JT, Wittman ME, Cooke RM, Lodge DM, Rothlisberger JD, Zhu X, Johnson TB (2015) Forecasting the impacts of silver and bighead carp on the Lake Erie food web. Transactions of the American Fisheries Society 145: 136–162. https://doi.org/10.1080/00028487.2015.1069211

Supplementary material 1

Table S1

Authors: Steven Crookes, Tej Heer, Rowshyra A. Castañeda, Nicholas E. Mandrak, Daniel D. Heath, Olaf L.F. Weyl, Hugh J. MacIsaac, Llewellyn C. Foxcroft

Data type: analytical table

Explanation note: ANOVA of eDNA detection levels (Cq) across sites and rivers.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/neobiota.56.47475.suppl1